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Detection and typing of STEC at the NRL NL

Annet Heuvelink

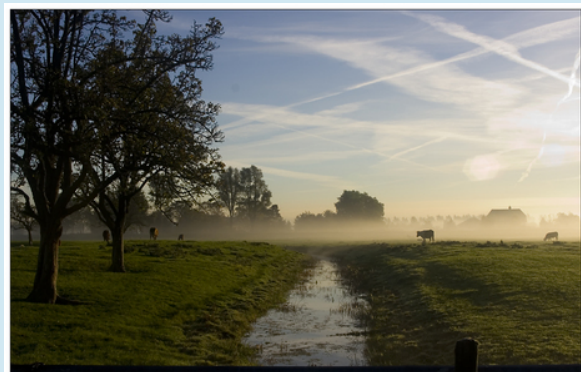
Food and Consumer Product Safety Authority (VWA), Zutphen, The Netherlands

NRL *E. coli* NL



Detection – samples

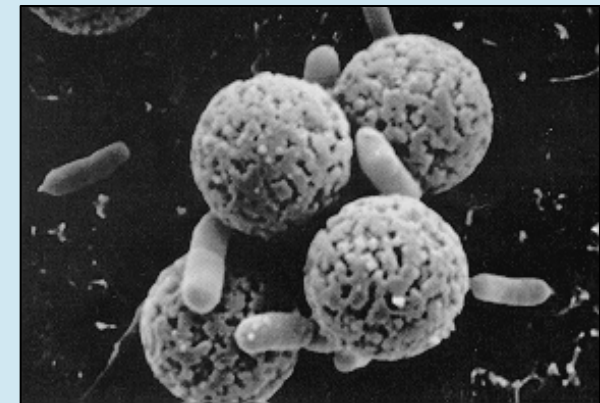
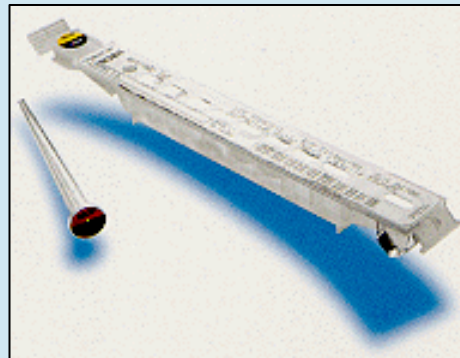
- Animal faeces
- Foods
- Environmental samples



Detection – methods for STEC 0157

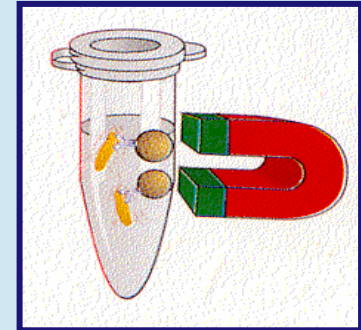
➤ Cultural methods

- Immunomagnetic separation and concentration (IMS) techniques
- VIDAS system



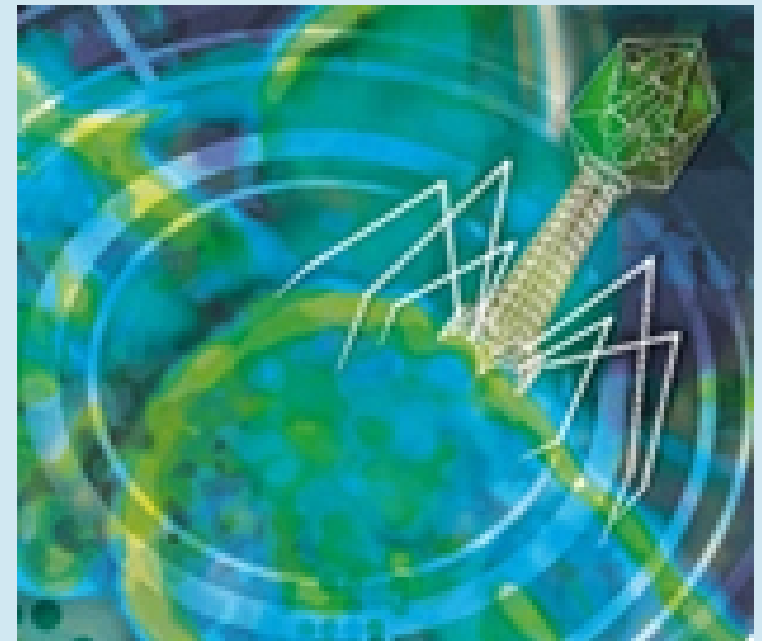
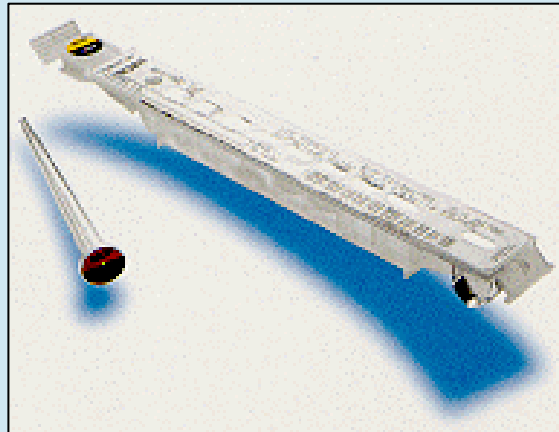
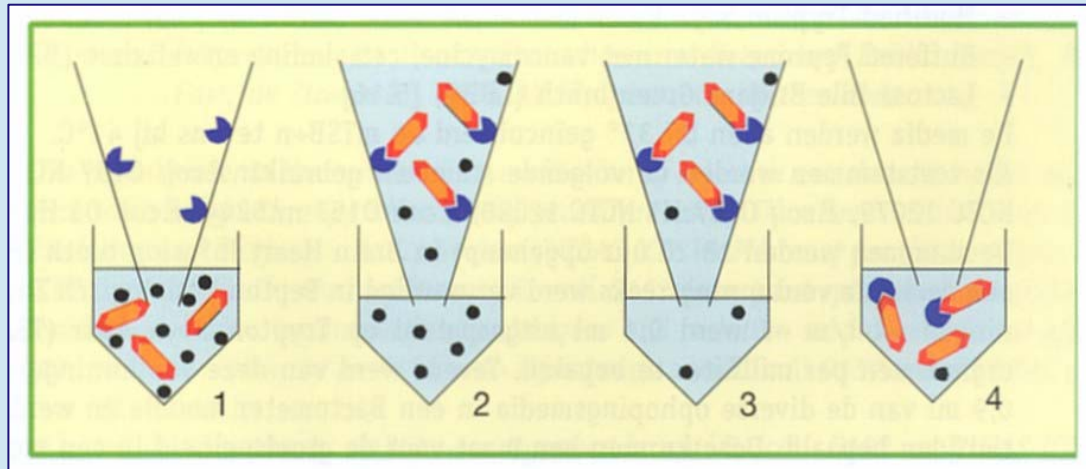
Isolation method *E. coli* O157 (ISO 16654:2001(E))

- **Selective enrichment:** - modified tryptone soy broth + novobiocin (mTSB+n)
(6 h and 18-24 h at 41.5°C)
- **Immunomagnetic separation and concentration (IMS)**
- **Isolation:** - cefixime tellurit sorbitol MacConkey (CT-SMAC) agar (50 µl)
(18-24 h at 37°C)
- 2nd selective agar of own choice (50 µl)
- **Confirmation:** - indole production / biochemical identification kit
- agglutination with *E. coli* O157 antiserum



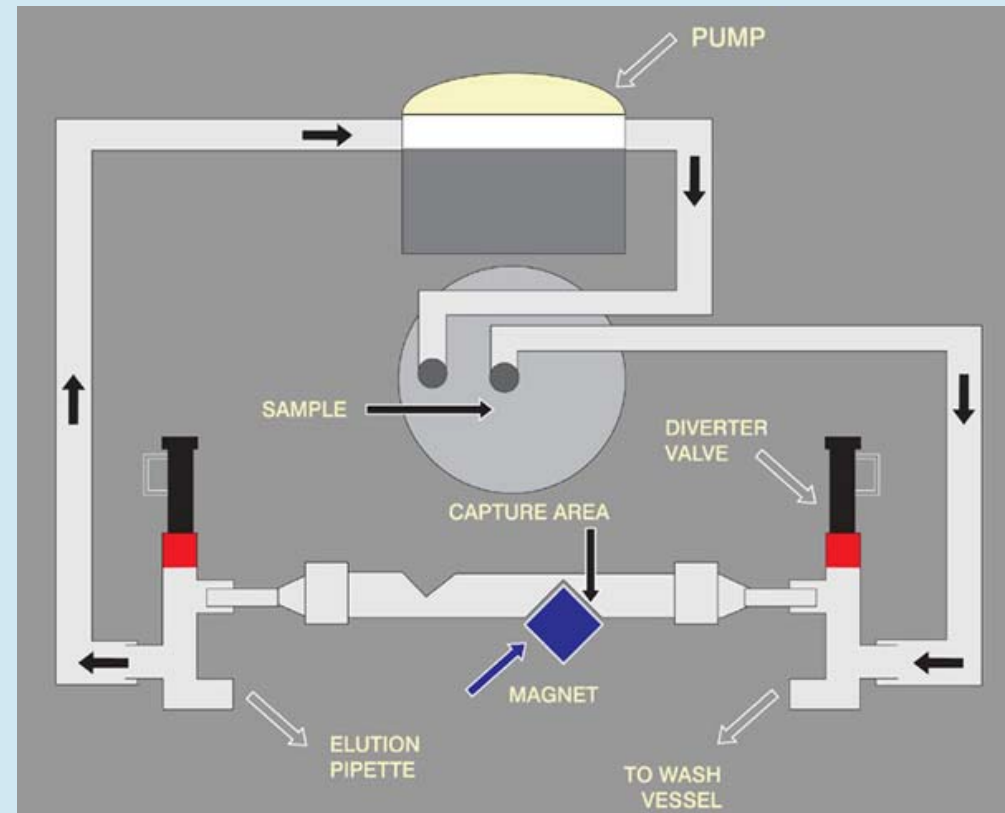
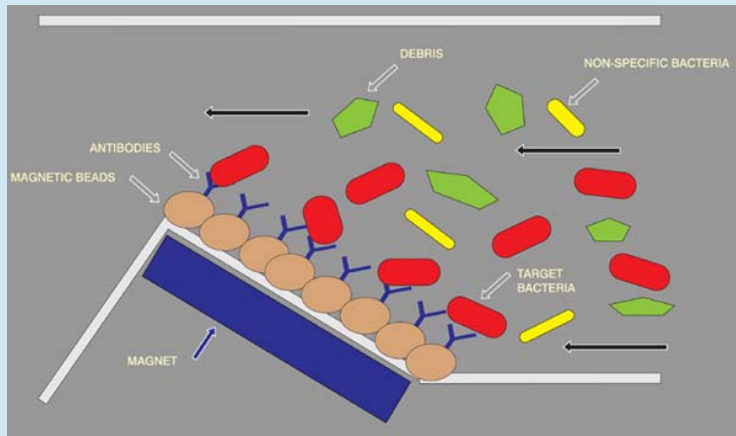


VIDAS immunoconcentration & VIDAS UP[®] *E. coli* O157:H7 (bioMérieux)



Recirculating immunomagnetic separation (RIMS)

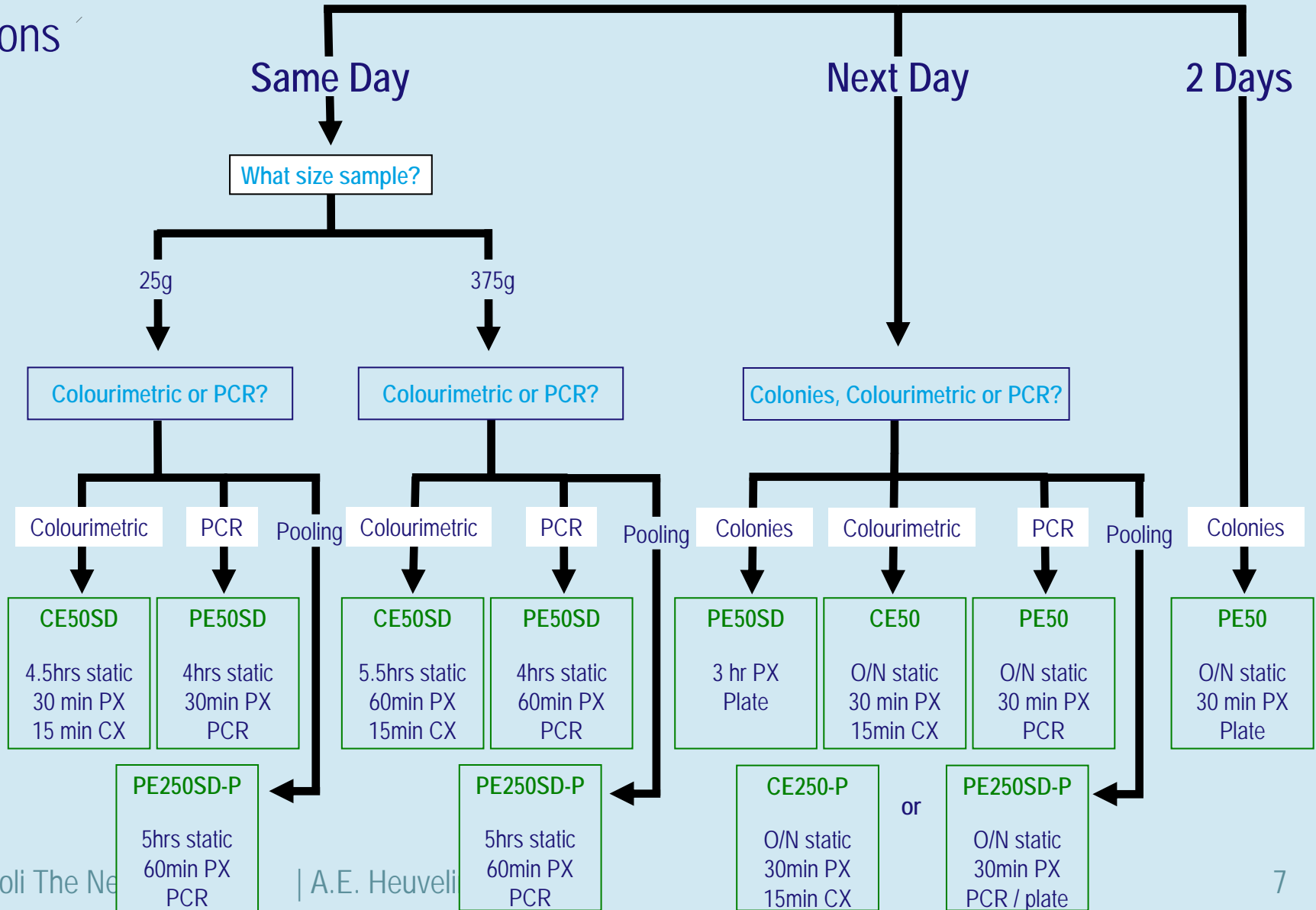
Pathatrix (Matrix Microscience)





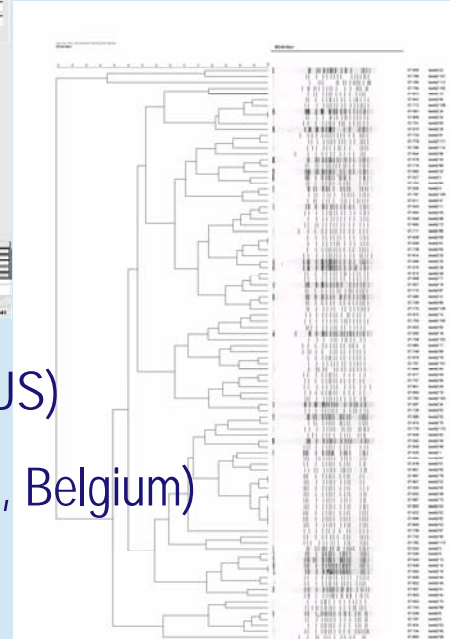
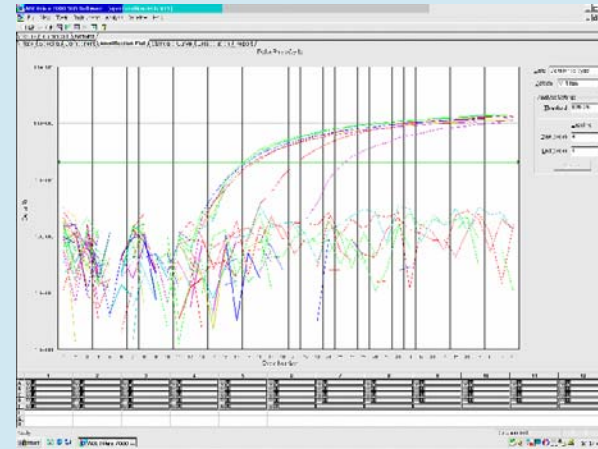
When would you like your result?

Test options



Typing of STEC 0157

- PCR : *stx1*, *stx2*, *eae*, *hly*_{EHEC}
- PFGE : - *Xba*I digestion of genomic DNA (PulseNet protocol, CDC, Atlanta, US)
- pattern analysis with BioNumerics software (Applied Maths, Kortrijk, Belgium)
- Phage typing (Laboratory for Enteric Pathogens of the Central Public Health Laboratory in London)
- *stx* subtyping, MLVA, MLST, SNP typing



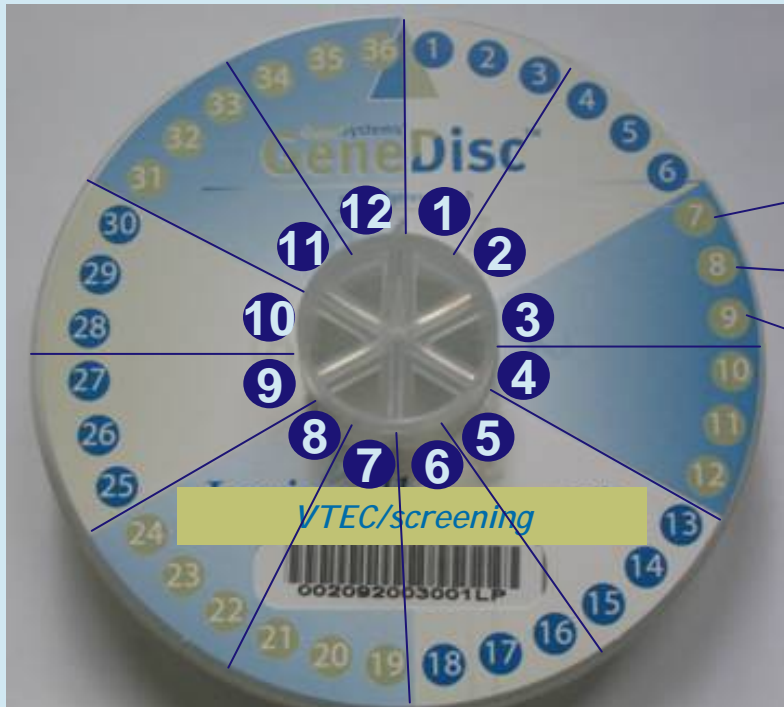


Detection – methods for VTEC non-O157

- Molecular methods
 - (real-time) PCR
- Cultural methods



Real-time PCR - GeneDisc



FAM

ROX

Flic H7

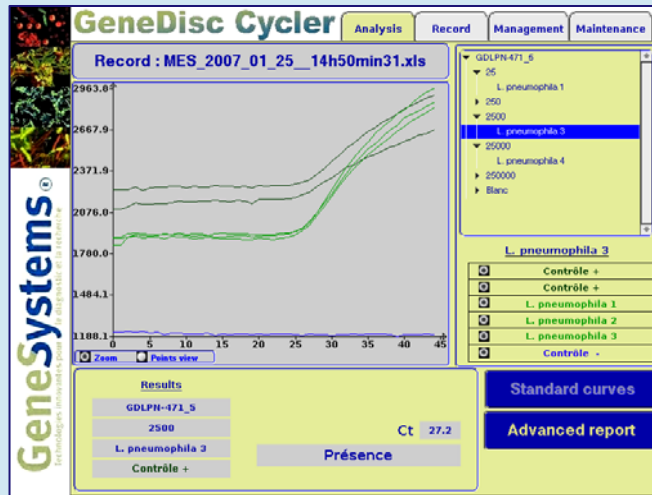
E. coli stx

rfbE O157

E. coli eae

Negative Ctrl

Inhibition Ctrl





Real-time PCR - GeneDisc – 3 Packs for VTEC

- O157 & STEC Screening GeneDisc Pack: *stx1* & *stx2*, *eae*, O157, *ehx*
- EHEC Identification GeneDisc Pack: O26, O103, O111, O145, H7
- Salmonella, O157 & STEC Screening GeneDisc Pack:
stx1 & *stx2*, *eae*, O157, *Salmonella*

Real-time PCR – GeneDisc – Our study design

- Samples: animal faeces & raw meats
- Enrichment: mTSB+n (41.5°C) & BPW (37°C)
- PCR screening
 - DNA extraction: Chelex-based
 - *stx1* & *stx2*, *eae*, O157, and *Salmonella* (GeneDisc & Light Cycler)
 - O26, O103, O111, O145, and H7 (GeneDisc & Light Cycler)
- Cultural method
 - VTEC O157: VIDAS-ICE and plating onto selective agar
 - *Salmonella*: selective enrichment using MRSV and plating onto selective agar



Real-time PCR - GeneDisc – Results

- animal faeces ↔ raw meats
- mTSB+n (41.5°C) ↔ BPW (37°C)
- GeneDisc ↔ Light Cyclor ↔ VIDAS ICE
- Different target genes



Detection - methods for VTEC non-O157

- Molecular methods

 - (real-time) PCR

- Cultural methods



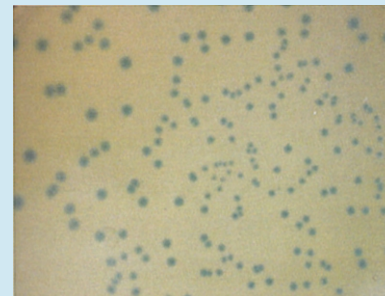
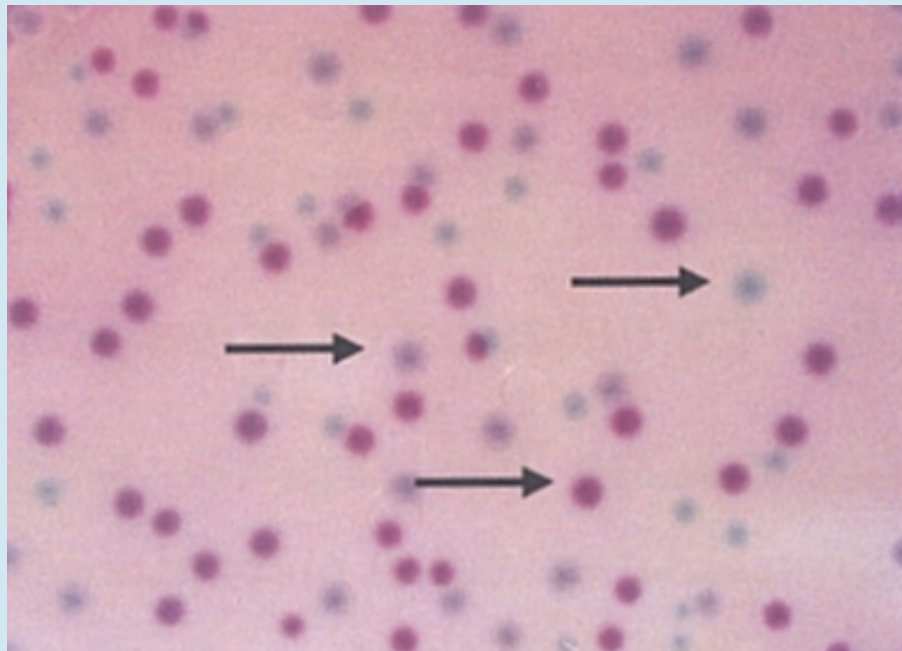
New selective differential media for STEC

Possé et al., FEMS Letters Microbiol 2008; 282:124-31

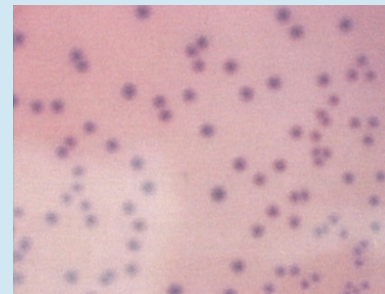
Serotype	Differentiation medium (D)			Confirmation medium (C)				
	D-sucrose	L-sorbose	FP% D (range)	D-arabinose	L-rhamnose	dulcitol	D-raffinose	FP% D+C (range)
O26	+	+	39 (32–47)	-	-	-	-	0.05 (0.04-0.06)
O103	+	-	45 (38–53)	-	-	-	-	0.3 (0.26-0.37)
O111	+	-	45 (38-53)	+	-	+	-	37.6 (31.8-44.3)
O145	-	-	8 (0–15)	+	-	-	-	1.008 (0.0–1.9)
Commensal <i>E. coli</i>	85% +	47% +		90% +	98% +	93% +	86% +	

New selective differential media for STEC

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O145 (green cfu)



O103 and O111 (blue cfu)



O26 (purple cfu)



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Thank you for your attention